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FURTHER STUDIES OF THE TOXIC SUBSTANCES OBTAINABLE FROM PNEUMOCOCCI.*

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Recently I have shown[1] that the appearance and then the disappearance of toxic substances in suspensions in salt solution of pneumococci and other bacteria, when kept at 37° C., are associated with proteolysis. These toxic substances, as measured in the guinea-pig, are very similar, and it makes no difference whether they are obtained by autolysis, by the action of normal or of immune serum, or by leukocytes *in vitro*. The symptoms caused are the same as those that appear in sensitized animals on reinjection of the unautolyzed extracts.

In the following I wish to present the results of further experiments on the toxic substances from pneumococci; on the conditions under which they appear and disappear in extracts and autolysates, in normal and immune serum mixtures, and in pneumococcus exudates; and on the relation they have to immunity in pneumococcus infections, and to anaphylaxis.

THE RELATIVE TOXICITY OF AUTOLYTIC EXTRACTS OF VIRULENT PNEUMOCOCCI FOR NORMAL AND SENSITIZED GUINEA-PIGS.

Although the difference in the behavior of normal and sensitized pigs to various pneumococcus extracts is mentioned in a previous paper a closer study of this point was thought worth while.

The pneumococci used in the experiment in Table 1 were grown in heated ascites-dextrose (0.2 per cent) meat-broth for 24 hours, washed once, and then suspended in salt solution so that 1 c.c. of the suspension contained approximately two and a half billion cocci. The virulence of the strain was such that one-half of the surface growth of a blood agar slant intraperitoneally killed a guinea-pig weighing 200 gms. in 16 hours. Disintegration of

* Received for publication May 25, 1912.

[1] *Jour. Infect. Dis.*, 1911, 9, p. 190; 1912, 10, p. 113.

pneumococci is directly proportional to the amount of proteolysis as indicated by formol titration.

The rotatory activity of this extract diminished and the amino nitrogen increased as the toxicity appeared and then disappeared. The results in Table 1 indicate strongly that the undigested pneumococcus protein is rapidly split into toxic material in the previously sensitized pig, and that the pneumococcus contains a ferment which splits its protein into similar cleavage products at a slower rate *in vitro* so that it becomes highly toxic for normal guinea-pigs and only slightly or not at all toxic to sensitized pigs. It seems as if the toxic material is rapidly split beyond the toxic stage in the sensitized animal. When the toxicity of clear pneumococcus

TABLE 1.

THE RELATIVE TOXICITY OF AUTOLYTIC EXTRACTS OF VIRULENT PNEUMOCOCCI FOR NORMAL AND SENSITIZED GUINEA-PIGS.

MATERIAL INJECTED	FORMOL TITRATION	LEVOROTATION IN MINUTES	TOXICITY—7 C.C. INJECTED INTO JUGULAR VEIN OF GUINEA-PIGS WEIGHING 200–225 GMS.	
			Normal	Sensitized
Virulent pneumococcus extract prepared at 37° C. for 5 hours	0.2 c.c. 0.5 c.c.	.. ..	No immediate symptoms. Death in 10 days	Death in 3 minutes from typical symptoms
Virulent pneumococcus extract prepared at 37° C. for 72 hours	0.3 c.c. 0.65 c.c.	12′ ..	Death in 2 minutes from typical symptoms	Slight symptoms, remained well
Virulent pneumococcus extract prepared at 37° C. for 5 days	0.3 c.c. 0.85 c.c.	2′ ..	No symptoms, remained permanently well	No symptoms, remained permanently well

extracts for normal pigs first appears the toxicity for sensitized pigs is greater than when the toxicity for normal pigs is at its height or is diminishing. Here the sensitized pigs often show no noticeable symptoms. Moreover, pneumococcus suspensions which have become toxic for normal pigs may be even more toxic for sensitized pigs. The undigested protein held by the partially autolyzed pneumococci must be the source of the toxic material. After proteolysis has gone still farther the toxicity for both normal and sensitized pigs disappears. The cause of the late death (10 days) of guinea-pigs (shown in Table 1, and observed many times before), which have received a larger dose of pneumococcus extract or suspension of pneumococci before the toxic stage is reached, is not

so clear; possibly it is due to sensitization so that undigested pneumococcus protein stored in the tissues is rapidly split into toxic material. The quantity of the acutely toxic substance in the circulation may not be great enough to provoke bronchial spasm. The late death is not observed in animals injected with autolysates which have become non-toxic. This explanation is in harmony with the work of Vaughan on egg white.

In this connection it should be stated that difference in the behavior of normal and sensitized pigs has been observed also with pneumococcus extracts which have been made toxic with immune serum and normal serum, with normal serum only, and with leukocytes; and also with salt solution extracts of pneumococcus exudates.

THE EFFECT OF NORMAL AND IMMUNE (SENSITIZED) GUINEA-PIG SERUM ON THE TOXICITY AND ROTATORY POWER OF PNEUMOCOCCUS EXTRACTS.

Extract 276, used in the experiments in Table 2, was prepared by suspending virulent pneumococci in NaCl solution for 24 hours

TABLE 2.

EFFECT OF SERUM ON PNEUMOCOCCUS EXTRACTS.

MIXTURES. 7 C.C. INJECTED INTO NORMAL GUINEA-PIGS WEIGHING 200 GMS.	LEVOROTATION AND TOXICITY			
	10 Minutes	2 Hours	4 Hours	24 Hours
Extract 276, 3 parts. Normal guinea-pig serum 1 part	1° 45′ No symptoms	1° 52′ No symptoms	1° 43′ No symptoms	1° 25′ Definite symptoms
Extract 276, 3 parts. *Immune guinea-pig serum, 1 part	2° 17′ Slight symptoms	2° 4′ Death in 4 minutes	2° 5′ Severe symptoms	1° 35′ No symptoms
NaCl sol., 3 parts. Normal guinea-pig serum, 1 part	2° 6′ No symptoms	2° 4′ No symptoms		
NaCl sol., 3 parts. Guinea-pig serum, 1 part	2° 9′ No symptoms	2° 4′ No symptoms		

* The guinea-pigs had been injected on two occasions with heat-killed virulent pneumococci.

at 37° C., ether being added and allowed to evaporate through the cotton plugs, and keeping for 48 hours in the ice-chest. Autolysis had gone on so that 7 c.c. produced severe but not fatal symptoms in a normal pig, and no symptoms in a sensitized pig of the same weight. The polariscopic reading of the extract at the time of the experiment was 5′ levorotation. The polariscopic readings were

made in a 10 cm. tube. While a constant relation between the rotatory power of mixtures of pneumococcus extracts and normal and immune serum, and their toxicity, cannot be made out, the appearance and disappearance of the toxicity are always associated with a diminished rotation. The amino nitrogen as determined by formol titration in mixtures of pneumococcus extract and serum (normal and immune) is practically the same before the toxic stage is reached and after it has disappeared. In other words, the polariscope here seems to be a better means of determining proteolysis than formol titration. The toxic substances here also seem to be intermediate products in the digestion of pneumococcus protein just as in the autolysates in NaCl solution. Its appearance and disappearance go hand in hand with evidences of proteolysis.

The action of normal guinea-pig serum on pneumococcus extracts has been studied further. The addition of serum to pneumococcus extracts before they have become toxic hastens the production of the toxic substance. Its addition during the height of toxicity may cause a diminution of the toxicity for a longer or shorter time, as is the case in the experiment given in Table 2. Serum added for the first time after the toxicity has disappeared by autolysis in NaCl solution rapidly restores the toxicity but not when added a second time. The action of sensitized guinea-pig serum on pneumococcus extracts is very similar except that its action is more rapid and often seems to yield more toxic material than normal serum.

From these observations the rapid parenteral digestion of pneumococcus proteins into identical toxic products in the sensitized animal on reinjection can scarcely be questioned. This conception would seem to afford the best explanation of the peculiar difference in the behavior of normal and sensitized pigs toward pneumococcus extracts and autolysates.

Dick[1] has shown that the proteolytic power of the serum toward pneumococcus protein is increased at the time of crisis in lobar pneumonia. This fact, together with the demonstration of the appearance and rapid disappearance of toxicity in immune serum, where there is greatest proteolysis, makes it seem likely that crisis

[1] *Jour. Infect. Dis.*, 1912, 10, p. 383.

in lobar pneumonia may be due in part to an increased proteolytic power of the serum on pneumococci.

MECHANISMS OF INTOXICATION WITH PNEUMOCOCCUS AUTOLYSATES AND OF INTOXICATION IN ANAPHYLAXIS COMPARED.

In order to determine further whether similar toxic substances are produced *in vivo* in immediate anaphylaxis as *in vitro* by autolysis of pneumococci, a series of experiments on normal guinea-pigs was made similar to those made by Auer and Lewis on sensitized guinea-pigs.

Briefly stated, the results obtained show that guinea-pigs which are deeply under the influence of ether, of chloroform, of urethan, or of morphin have much less or no bronchial spasm even when approximately one and one-half times the minimum but regularly fatal dose is injected. The anesthetics do not prevent death, however. Death here usually occurs in from 30 minutes to two hours. Some dyspnea is usually observed, then breathing becomes shallow, the animal is prostrated, and the temperature and leukocytes drop markedly. Pulmonary and more particularly cardiac, intestinal, and adrenal hemorrhages are often marked. Atropin in proper doses prevents bronchial spasm, just as Auer and Lewis[1] found in anaphylaxis, and as Mita[2] recently found in acute beef serum intoxication.

When 0.08 mg. of atropin sulfate is injected intravenously just before the extract, it prevents bronchial spasm completely even when one-third more than the regularly fatal dose of the extract is given; but when the same dose of atropin is injected immediately after the extract it fails to protect. The protective power if injected 15 minutes earlier is very much less than when injected immediately before the extract. The animals which are thus protected against the immediate death usually die over night. A comparison of the effect of ether and atropin when the same toxic extract is injected shows that the amount of hemorrhage is greater in the animals receiving ether than in the animals receiving atropin. The former die inside of two hours and the latter in 18 to 24 hours.

[1] *Jour. Exp. Med.*, 1910, 12, p. 151.

[2] *Ztschr. f. Immunitätsf.*, 1911, 11, p. 501.

It has been shown by Auer and Lewis also that the bronchial spasm in immediate anaphylaxis is due to peripheral action. If it could be shown that toxic pneumococcus autolysates have a similar peripheral action it would be evidence that the toxic substances in the two instances are similar. Accordingly the effect of a highly toxic pneumococcus autolysate was tested upon pithed normal guinea-pigs. The animals were partially anesthetized with ether, a glass canula was tied in the trachea, the vagi cut, and after artificial respiration was begun, the medulla, the lower brain, and the cord were destroyed by pithing. The blast of air, which was constant in volume and rate throughout the experiment, produced a well defined expansion of the chest. Enough time was allowed to elapse so that the effect of the ether had worn off. The doses selected were one and one-half times and twice the minimum fatal dose for untreated pigs of the same size (250 gms.). The injection (12 c.c.) was made into the jugular vein. Two to four minutes after the injection the chest expansion grew less and less, the chest became fuller until no oscillations could be made out between the blasts of air driven into the trachea. On opening the thorax the heart was found beating and the lungs distended and pale. A good sized slit (0.5 cm.) in the margin of the lung showed no escape of air with forced injections, and no bleeding. Pressure on the excised lung failed to collapse it and no bubbles of air escaped from the trachea when pressure was made under water, presenting an exactly similar picture to the lung in the unpithed animal and in the sensitized animal on reinjection. The air is imprisoned in the lung.

Adrenalin, 0.2 to 0.1 mg., when injected intravenously just before, together with, and immediately after toxic pneumococcus autolysate, prevents death from asphyxia by relieving the bronchial spasm, the animals usually dying soon after with marked pulmonary and other hemorrhages. The action of adrenalin is similar to that found by Jonuschke and Pollak[1] in cats following muscarin injection, and by Pal[2] in guinea-pigs following peptone injections.

Biedl and Kraus[3] were unable to protect anaphylactic guinea-pigs and normal guinea-pigs injected with peptone by forced

[1] *Arch. f. Exp. Path. u. Pharm.*, 1911, 66, p. 205.

[2] *Deutsche med. Wchnschr.*, 1912, 38, p. 5.

[3] *Ibid.*, 1911, 37, p. 1300.

tracheal respiration, whereas they were able to protect against acute death following injection of "anaphylatoxin," and hence they draw the conclusion that the mechanism of the latter must be different from that of peptone. I have made a similar study of the effect of forced tracheal respiration in normal and sensitized guinea-pigs. The results briefly stated are these: Forced respiration protects both normal and sensitized guinea-pigs against approximately one-fourth more than the minimum fatal dose of toxic pneumococcus autolysate in the normal, and of unautolyzed pneumococcus extracts in the sensitized animals. But against larger doses it fails to protect in each case. Hence it is clear that the protective power of this procedure is in both cases a matter of degree.

Finally, repeated tests show conclusively that injections into the left heart of toxic pneumococcus autolysates require a considerably larger dose to kill normal guinea-pigs regularly than injections into the jugular vein or right heart, whereas in sensitized animals the reverse is true. This fact is in harmony with the idea that there occurs a rapid digestion of the unautolyzed extract into toxic material in the sensitized animal, injection into the left heart affording greater opportunity for enough material to be formed to produce bronchial spasm.

In this connection another point which has been observed should be mentioned. While it requires a larger dose of pneumococcus autolysates to produce fatal bronchial spasm on left heart than on jugular injection the systemic spasms are more pronounced.

The symptoms produced, the condition of the lungs after death, and the facts brought out here show clearly that death following injections of toxic pneumococcus autolysates is due to asphyxia from bronchial spasm, as in anaphylaxis. The results speak strongly in favor of the view that similar toxic products are concerned in each case.

In this connection it should be stated that while the serum of guinea-pigs dying in immediate anaphylaxis (horse serum) has been found to produce the milder symptoms of anaphylaxis like those following non-fatal injections of toxic pneumococcus autoly-

sates—dyspnea, irritability, drop in temperature and leukocytes—it has not been possible to produce fatal bronchial spasm.

RESTORATION OF TOXICITY OF AUTOLYZED PNEUMOCOCCUS EXTRACTS.

Extract 258, used in the experiments in Table 3, was prepared in the usual way by suspending pneumococci in NaCl solution at 37° C. and testing the toxicity at intervals by injecting 7 c.c. into the jugular vein. It produced no immediate symptoms when first made, definite symptoms at the end of 24 hours, death in 4 minutes at the end of 48 hours, and again no symptoms at the end of 96 hours. The experiments were made after this extract had been kept in the ice-chest for 6 months; 8 c.c., and 6 c.c. to which 2 c.c. of NaCl solution were added, now produced no symptoms. It is

TABLE 3.

RESTORATION OF TOXICITY OF PNEUMOCOCCUS AUTOLYSATES.

Mixtures	Symptoms*
Extract 258, 6 c.c.+normal guinea-pig serum, 2 c.c. at once	No symptoms
Extract 258, 6 c.c.+normal serum, 2 c.c. after 1 hour at 37° C.	Death in 3 minutes
Extract 258, 6 c.c.+normal serum after 2 hours at 37° C., then in ice-chest, 48 hours	No symptoms
Extract 258, 6 c.c.+normal serum at 37° C. 2 hours and 1 hour after adding fresh serum a second time†	No symptoms
Extract 258, 6 c.c.+normal serum 2 c.c. heated (60° C. one-half hour) 37° C. 1 hour	Definite symptoms
Extract 258, 6 c.c.+2 c.c. serum+charcoal 1 gm. 1 hour at 37° C.	Slight symptoms
Extract 258, 6 c.c.+2 c.c. normal serum treated with charcoal (10 gms. per c.c. serum at 37° C. 1 hour), then at 37° C. 1 hour	No symptoms
Extract 258, 6 c.c.+charcoal (1 gm. per c.c. at 37° C. 1 hour)+2 c.c. normal serum, then at 37° C. 1 hour	No symptoms
Extract 258, 6 c.c.+2 c.c. normal serum which has been extracted with ether, then at 37° C. 1 hour	No symptoms
Extract 258, 6 c.c.+2 c.c. ether extracted serum to which the equivalent amount of ether soluble substance is again added, at 37° C. 1 hour	No symptoms

* Control experiments, in which corresponding mixtures of serum and NaCl solution were injected, failed to give symptoms.

† A closer study of the effect of adding serum a second time to reactivated extracts by injecting the mixtures at various intervals shows that the serum now fails entirely to cause a return of toxicity.

shown in Table 3 that normal guinea-pig serum reactivated temporarily the toxicity of pneumococcus autolysates which had lost their toxicity. The second addition of serum after the toxicity has disappeared no longer has this power. It would seem as if there had occurred a certain equilibrium in the autolysate so that the autolytic ferment would no longer convert into toxic material fractions of the pneumococcus protein which, however, are acted on under the new conditions. The active principle which makes new

toxic material in normal serum seems to be complement, because it is theromolabile, adsorbed by animal charcoal, and removed and destroyed by ether. The material from which normal serum makes this toxic substance is also removed by animal charcoal. That mixtures of heated serum and pneumococcus extract become toxic has been observed and deserves further study because it suggests that here toxic material may be produced by proteolytic action of the extract on the heated serum.

The observation that a leukocytic pneumococcus extract which has become non-toxic becomes toxic in 4 hours on the addition of new pneumococci is in favor of the view that the proteolytic action of these pneumococcus leukocytic extracts play a definite rôle in the production of toxic substances. Moreover, if pneumococcus autolysates which have become non-toxic are heated, it takes longer for normal serum to render them toxic again, and at times it fails entirely to do so.

TABLE 4.

RESTORATION OF NON-TOXIC LEUKOCYTIC EXTRACTS OF PNEUMONIC LUNGS BY NORMAL SERUM.

The Mixtures Were Kept at 37° C. 7 Hours and on Ice 20 Hours before Injection	Symptoms in Normal Guinea-Pigs Following Jugular Injection of 7 c.c.
Extract "A,"* 4 c.c.+normal guinea-pig serum, 2 c.c.	Death in 4 minutes
Extract "A," 4 c.c. heated 60° C. 1 hour+normal guinea-pig serum, 3 c.c.	Very slight symptoms
Extract "A," 4 c.c.+heated (60° C. 1 hour) normal guinea-pig serum, 3 c.c.	Definite but mild symptoms
Extract "A," unheated 3 c.c., heated 1 c.c.+normal guinea-pig serum, 3 c.c.	Severe symptoms but recovered

* Extract "A" was prepared as indicated below in Table 5, after being kept at 37° C. for 3 days.

VARIATIONS IN THE SUSCEPTIBILITY OF GUINEA-PIGS TO TOXIC PNEUMOCOCCUS AUTOLYSATES.

Jugular injections.—Guinea-pigs vary considerably in their susceptibility to the toxic action of pneumococcus autolysates. This is especially true of those which have been fed carrots. The difference in susceptibility is much less marked in guinea-pigs which are starved, and the general susceptibility is greater. It has been found that the minimum fatal dose for guinea-pigs which have been starved for 60 hours is approximately one-third less than that required for well fed pigs of the same weight. For these reasons it

has been the rule not to feed the animals greens, especially carrots, on the day of the experiments. Other food, such as oats and hay, makes less difference.

The study which has been made of the altered susceptibility of guinea-pigs following various injections may be summarized as follows: Filtrates of broth cultures of pneumococci, autolyzed extracts, both before and especially after the toxic stage, and to a lesser degree autolyzed pneumococci, render guinea-pigs insusceptible to doses of pneumococcus autolysates, otherwise fatal, in 2, 4, 6, 24, and 48 hours. The animals do not have bronchial spasm and remain permanently well. The control injections of broth, NaCl solution, and of extracts of typhoid bacilli do not render guinea-pigs insusceptible to pneumococcus autolysates.

Portal injections.—The power of the liver to neutralize various poisons is well known. The close relation of the liver to infection in general and more particularly to bacterial intoxications, which have their origin in the intestinal tract, as well as the fact that typhoid bacilli and colon bacilli have been found in my experiments to yield on autolysis an exactly similar toxic material to that yielded by pneumococci, seems to indicate that the effect of portal injections of highly toxic autolysates should be studied.

Experiments were made under ether and urethan anesthesia by injecting the material into the main branch of the portal vein, but the results were so unsatisfactory that a different method was used. This consisted in making a small incision in the abdomen from 0.5 to 1 cm. in length, and injecting the toxic dose into a small branch of the mesenteric vein of the small intestines. The needle employed was of the same caliber as the one for jugular injections. The results obtained are striking. Well fed guinea-pigs fail to develop the acute symptoms even when one and one-half times the minimum fatal dose by the jugular is injected. The animals are made only slightly ill. Starved guinea-pigs (48 to 60 hours), on the other hand, react almost as promptly as on jugular injection, and with typical symptoms. Two hours after they have eaten ravenously of carrots, the acute symptoms are again very much reduced, and in 24 hours after feeding they again behave as normal pigs.

Complement titration and opsonic determination of the serum from normal pigs and from starved pigs fail to show a measurable difference in complement content and in opsonins for pneumococci. The late toxic action in the starved animals was also strikingly greater than in those well fed, although here the liver was not always sufficient to protect the well animals against a fatal outcome.

In other words, the protective power of the liver against toxic bacterial products may be related in some way to its glycogenic function. It is possible, however, on the other hand, that this mechanism is related to the digestive function. These experiments would seem to explain why great fatigue and hunger render the human, especially children, more susceptible to intoxication, particularly of intestinal origin. It is clear that the behavior of the starved animals toward toxic pneumococcus autolysates does not depend on the complement content of the serum.

Because fresh normal serum has the power to restore toxicity to autolysates which have lost it, the effect of extreme hemorrhage on the susceptibility of otherwise normal pigs was also studied. Four normal guinea-pigs were injected with a known toxic autolysate after having been bled varying amounts by cutting the carotid artery. Two were injected intraperitoneally with NaCl solution, and were bled until the hemoglobin was 20 per cent. The other two were bled nearly to death. All four animals died from typical symptoms in the usual way, the symptoms if anything being more marked than usual. Hence the conclusion seems warranted that the toxic substance exists in the autolysate, and is not made on injection by the action of complement, and that guinea-pigs are more susceptible to the action of the substance after acute hemorrhage.

OBSERVATIONS ON THE TOXICITY OF PNEUMOCOCCUS EXUDATES, ETC.

The question whether or not toxic material is produced *in vivo* in pneumococcus infections similar to that *in vitro* on autolysis of pneumococci, and whether it disappears under similar conditions, was taken up also. A study of the blood of guinea-pigs dying from pneumococcemia, of the peritoneal exudate in guinea-pigs with pneumococcus peritonitis, of pus in pneumococcal empyema, and

of consolidated pneumonic lungs brings out the fact that in all these cases there is produced similar toxic material. Thus the serum of guinea-pigs dying from pneumococcemia, while it has never been found to contain enough free toxic material to produce bronchial spasm in normal guinea-pigs when first obtained, may produce fatal shock in normal pigs in 4 c.c. doses when kept at 37° C. for from 6 to 24 hours, and fatal immediate anaphylaxis in sensitized pigs as soon as drawn.

A portion of a consolidated lung in gray hepatization, still quite warm, which contained very many pneumococci, some well preserved and many in various stages of disintegration, was ground up with a meat chopper, strained through a thick layer of gauze after adding enough NaCl solution so that the strained material was of a thick syrupy consistence. This suspension was grayish in color, and was diluted with eight times its volume of NaCl solution, thoroughly shaken, and injected at once into the jugular vein of a normal and a sensitized pig of the same weight. The normal pig died in 5 minutes, the sensitized in 3 minutes, of typical symptoms. Ether was added to one portion of this suspension, which was then placed at 37° C. for three days. Injection of this material into two normal pigs was not followed by acute symptoms. Another portion, 250 c.c., was washed in NaCl solution resuspended in NaCl solution, and divided into two equal parts (A) and (B). To (A) virulent pneumococci from 300 c.c. of broth culture were added. To both parts 10 c.c. of ether were added and allowed to evaporate through the cotton plugs. The toxicity of these mixtures was then tested as indicated in Table 5. Cultures on blood agar made at the time of the injection proved sterile.

From the tests with the unwashed and washed lung extracts it is clear that there is produced in pneumonic lungs a highly toxic substance. That the pneumococcus plays an essential rôle in the formation of this acutely toxic substance is shown by the fact that when new pneumococci are added [(B) Table 5] the toxicity for both normal and sensitized pigs disappears later than when no new pneumococci are added [(A) Table 5]. Moreover, these extracts, consisting, as they do, chiefly of leukocytes and pneumococci, rapidly make toxic material from new pneu-

mococci after their toxicity has disappeared, as indicated in the last two experiments.

From these results we see that the same peculiar difference in the behavior of normal and sensitized pigs exists toward extracts of pneumonic lungs as to pneumococcus extracts in NaCl solution.

TABLE 5.

TOXICITY OF SUSPENSIONS OF PNEUMONIC LUNG MIXED WITH VIRULENT PNEUMOCOCCI.

MIXTURES	APPEARANCE OF PNEUMOCOCCI	SYMPTOMS IN GUINEA-PIGS (7 C.C. INTO JUGULAR VEIN)	
		Normal	Sensitized
(A) After 30 minutes at room temperature	Mostly gram positive Some gram negative	No symptoms	Death in 6 minutes
(B) After 30 minutes at room temperature	Mostly gram positive Some gram negative	Severe symptoms	Death in 3 minutes
(A) After 20 hours at 37° C.	Many gram negative	Severe symptoms. Nearly dead in 3 minutes	No symptoms
(B) After 20 hours at 37° C.	Many gram negative	Death in 3 minutes	Very severe symptoms. Nearly dead in 10 minutes
(A) After 48 hours at 37° C.	Nearly all gram negative	Slight symptoms	No symptoms
(B) After 48 hours at 37° C.	Nearly all gram negative	Severe symptoms. Nearly dead in 10 minutes	Slight symptoms
(A) After 72 hours at 37° C.	Nearly all gram negative	No symptoms	
(B) After 72 hours at 37° C.	Nearly all gram negative	Slight symptoms	No symptoms
(B) After 96 hours at 37° C.	Nearly all gram negative	No symptoms	No symptoms
(A) After 1 hour at 37° C. +unautolyzed vir. pn. from 6 c.c. broth at 37° C. for 4 hours	Both gram negative and gram positive	Severe symptoms, including bronchial spasm, but recovered	
7 c.c. NaCl sol. unautolyzed vir. pn. from 6 c.c. broth at 37° C. 4 hours	Nearly all gram positive	No symptoms	

The appearance and disappearance of the toxicity, however, occur earlier in the leukocytic pneumococcus mixtures. Similar results have been obtained in dogs.

A similar study of extracts in NaCl solution of consolidated lungs (for which I am indebted to Dr. Le Count) shows that the amount of toxic substance which kills guinea-pigs by provoking bronchial spasm, while present at all stages of consolidation, is greatest in those which contain mucoid exudate and from which smears and cultures show the presence of the greatest number of pneumococci as well as the greatest evidence of disintegration of pneumococci and leukocytes.

Control experiments in which extracts in NaCl solution of normal lungs were prepared show that the amount of lung tissue must be larger and that the symptoms produced are not typical, death occurring somewhat later than when extracts of pneumonic lungs were used. This result is in harmony with the results of Dold[1] who found that extracts of various organs may be toxic on intravenous injection.

SUMMARY.

The toxic substances obtainable from pneumococci on autolysis in NaCl solution, from pneumococcus-leukocyte mixtures, from pneumococcus exudates, from the action of normal and immune serum on pneumococci, produce identical symptoms in normal guinea-pigs, and these symptoms are indistinguishable from those of immediate anaphylaxis. Postmortem appearances are also similar. The serum of sensitized guinea-pigs produces the toxic material from pneumococcus extracts more rapidly than normal serum and probably also in larger amounts. This is associated with a more rapid proteolysis as measured by the polariscope.

Morphin, ether, urethan, atropin, and adrenalin protect normal guinea-pigs against the toxic material, obtained *in vitro* from pneumococci, and also sensitized guinea-pigs on reinjection.

Forced respiration fails to protect in each instance when the doses are properly gauged.

The action of the toxic substances on normal guinea-pigs is peripheral, as in immediate anaphylaxis.

The behavior of normal and sensitized guinea-pigs toward unautolyzed extracts of pneumococci, which are non-toxic to the former and very toxic to the latter, toward partially autolyzed extracts, which are very toxic to the former, and slightly or not at all to the latter, and toward more completely autolyzed extracts, which are non-toxic to both, speaks strongly in favor of the view of a rapid parenteral digestion into toxic cleavage products in sensitized animals.

The serum obtained from guinea-pigs in anaphylactic shock has been found to contain substances which produce symptoms

[1] *Ztschr. f. Immunitätsf.*, 1911, 10, p. 53.

in normal pigs indistinguishable from the milder symptoms of anaphylaxis and from those following injections of non-fatal doses of pneumococcus autolysates.

In view of these facts the conclusion seems warranted that the symptoms in anaphylaxis are due to toxic protein cleavage products, formed rapidly after reinjection, and identical with the toxic products obtained *in vitro*.

Normal guinea-pigs vary considerably in their susceptibility to jugular injections of toxic pneumococcus autolysates just as they do to intoxication on reinjection of proteins. When starved they are more susceptible and the individual variations are less marked. Well fed guinea-pigs are quite insusceptible to portal injections, while those starved are nearly as susceptible to portal injections as to jugular injections.

After pneumococcus autolysates have become non-toxic, their toxic power may be restored by the addition of normal or immune serum, by leukocytes, by the addition of unautolyzed pneumococci, and by extracts. The addition of serum or leukocytes a second time, however, fails to restore the toxicity unless new extract is added.

The appearance and disappearance of the toxic substances seem to be definitely related to proteolysis. This is true alike in pneumococcus autolysates, in serum mixtures, in leukocytic mixtures, and in pneumococcus exudates.

A single intravenous injection of non-fatal doses of extracts before they have become toxic, while highly toxic, and especially after the toxic stage is passed, or of autolyzed pneumococci, renders guinea-pigs insusceptible to subsequent injections of toxic pneumococcus autolysates.

Recovery from pneumococcus infections, the crisis in lobar pneumonia, for example, probably occurs when the toxic substances of the pneumococci have been digested beyond the toxic stage. The factors which would seem to be concerned in this process are the autolytic ferment of the pneumococcus, the increased proteolytic power of the serum, the proteolytic action of the leukocytes, and the increased opsonic power of the serum, with a consequent greater phagocytosis.